REMARKS

By this amendment, the specification has been amended to correct minor clerical errors and to incorporate the Substitute Sequence Listing submitted herewith.

In particular, the paragraph at p. 20, ll. 3-17 of the specification has been amended to correct the spelling of the term "Creutzfeldt-Jakob"; the paragraph at p. 47, ll. 11-22 of the specification has been amended for clarity and to correct spelling errors; the paragraph at p. 68, ll. 6-25 of the specification has been amended to correct the spelling of the term "Friedrich's"; and the table at p. 71, l. 13 has been amended to correct the spelling of the term "Guillain Barre."

In addition, the paragraphs at p. 9, l. 27 to p. 10, l. 5 and at p. 42, ll. 1-23 of the specification have been amended to correct typographical errors and to update the reference to the U.S. patent application that was identified as Attorney Docket No. KW00-009C02-US to state that it has been accorded Application Serial No. 10/188,905. A copy of the filing receipt for Application Serial No. 10/188,905 is attached as Exhibit A. As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

The specification at p. 42, ll. 1-23 has also been amended to add the language "in which *in vivo* biological activity was determined by the polycythemic mouse bioassay." This amendment is supported by the application as originally filed at p. 42, ll. 10-11, which incorporates by reference Satake *et al*; 1990, *Biochim. Biophys. Acta* 1038:125-9 in its entirety for its description of molecular biological techniques for generating erythropoietin derivatives and testing them for *in vivo* biological activity of erythrocytes (*i.e.*, erythropoietic activity) using the polycythemic mouse bioassay. See Satake, page 126, col. 2: "in vivo biological activity was determined by the exhypoxic polycythemic mouse bioassay," citing Cotes and Bangham, 1961, Nature 191:1065-1067 (reference C102 of record in the subject application). As such, no new matter has been added with this amendment. See M.P.E.P. 608.01(p).

Lastly, sequence identifiers were inserted into the paragraphs at p. 39, l. 8 to p. 40, l. 14 and p. 89, ll. 14-20. In the paragraphs at p. 90, ll. 4-10, the sequence identifier for full-length erythropoietin (EPO) has been amended from SEQ ID NO:10 to SEQ ID NO:213.

Claims 1-70 were pending in the instant application. Claims 1-53, 59-68, and 69-70 are withdrawn from consideration. By this amendment, claims 54-58 are amended and new claims 71-73 are added, for the reasons discussed below.

Claims 54 and 57 have been amended to specify that the mutein recombinant tissue protective cytokine (alternatively referred to herein as "EPO mutein") comprises the amino acid sequence of SEQ ID NO:10 with a substitution of an amino acid residue at one or more of the following positions: (i) 11 to 15 [SEQ ID NO:1]; (ii) 44 to 51 [SEQ ID NO:2]; (iii) 100 to 108 [SEQ ID NO:3]; or (iv) 146 to 151 [SEQ ID NO:4]. Support for this amendment may be found in the claims and specification as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 40, ll. 3-7; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed. Claims 54 and 57 have also been amended to recite specific tests for determining erythropoietic and tissue protective activity. In particular, the recited muteins have: (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay, and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay. Support for this amendment may be found in the specification *inter alia* at p. 42, paragraph beginning at l. 1 as amended herein; Example 3 at p. 103, l. 18 to p. 104, l. 3; and Example 15 at pp. 124-125. In addition, claims 54 and 57 have been amended to recite that "an effective amount" of a pharmaceutical composition is administered. Support for this amendment may be found in the specification, *e.g.*, at p. 19, ll. 1-19.

Claim 58 has been amended to add the term "is at risk for." Support for this amendment may be found in the specification at, *inter alia*, p. 20, ll. 3-8; p. 24, ll. 16-19; p. 31, ll. 26-30; p. 64, ll. 13-15 and ll. 29-32; p. 79, ll. 19-25; and p. 80, ll. 6-15. The listing of indications in claim 58 has been amended to more distinctly point out the invention. Support for this amendment may be found in the specification, *e.g.*, at p. 20, ll. 3-17; p. 31, ll. 21-30; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; and p. 79, ll. 14-25. Claim 58 has also been amended to correct the spelling of Creutzfeldt-Jakob disease.

Claims 54 and 57 have also been amended to correct minor typographical and/or grammatical errors and to clarify the invention. Claim 56 has been amended to depend from claim 54. Claim 69 has been amended so that it is consistent with antecedent claim 57 as amended.

Support for new claims 71-73 may be found in the specification and claims as filed, *inter alia*, at p. 4, ll. 20 to p. 5, l. 4; p. 6, ll. 15-16; p. 6, l. 22 to p. 9, l. 20; p. 19, ll. 1-19; p. 20, ll. 3-17; p. 24, ll. 16-19; p. 31, ll. 21-30; p. 40, ll. 3-7; p. 42, paragraph beginning at l. 1 as amended herein; p. 64, ll. 13-15 and ll. 29-32; p. 66, l. 25 to p. 68, l. 32; the Table at pp. 69-71; p. 79, ll. 14-25; p. 80, ll. 6-15; Example 3 starting at p. 89, particularly p. 90, ll. 4-10 and p. 101, l. 19 to p. 102, l. 24; Example 15 at pp. 124-125; Example 16 at pp. 125-126; and Example 18 at pp. 128-129; the Substitute Sequence Listing for the corresponding sequences referred to in the aforementioned pages; and claim 2 as originally filed.

No new matter has been added by this amendment. Thus, claims 1-73 will be pending upon entry of the present amendment, and claims 54-58 and 69-73 will be under examination.

I. STATEMENT OF THE SUBSTANCE OF THE INTERVIEW

Applicants thank Primary Examiner Aditi Dutt and Supervisory Patent Examiner Jeffrey Stucker for the courtesies extended during the interview of February 6, 2008 at the United States Patent and Trademark Office ("the Interview"). Also present at the Interview were Drs. Anthony Cerami and Michael Brines, two of the inventors of the instant application, Frederick J. Hamble, Esq., of Warren Pharmaceuticals, Inc., Mary Catherine DiNunzio, Esq., of H. Lundbeck A/S, and Applicants' representatives Laura A. Coruzzi, Esq., Eileen E. Falvey, Esq., and Tracy J. LaGrassa, Ph.D., of Jones Day.

During the Interview an overview of the invention was presented. In summary, the inventors described their discovery that EPO provides tissue protective activity via a pathway distinct from the pathway it uses for erythropoiesis, *i.e.*, red blood cell production. EPO exerts its erythropoietic effect via an EpoR homodimer (the "Classical EPO Receptor"), whereas EPO's tissue protective activity is mediated through its interaction with a different receptor, referred to herein as the "Tissue Protective Receptor Complex," which is a

heteromer of EpoR and the beta common receptor (βcR). EPO signaling through the Classical EPO Receptor (present predominantly on blood forming cells) elicits an increase in erythrocytes, platelets, and blood pressure. In contrast, activation by EPO of the Tissue Protective Receptor Complex (present on many tissues) leads to a wider range of tissue protective effects. Based on the inventors' discovery that EPO can cross tight endothelial cell barriers, they found that exogenously administered EPO is capable of conferring tissue protection on any tissue that expresses the Tissue Protective Receptor Complex. Therefore, as a tissue protective cytokine, EPO counteracts the tissue damage at the root of a wide variety of diseases and disorders. For example, the inventors found that EPO protects against tissue damage caused by the pro-inflammatory cytokine TNFα, reduces the extent of TNFα-induced damage, and promotes healing and regeneration of affected tissues. Thus, the ability of EPO to prevent cell death and tissue damage that is common to many disease conditions demonstrates the widespread therapeutic implications of EPO and EPO muteins.

The inventors found that modified EPOs that cannot bind to the Classical EPO Receptor and stimulate red blood cell production were still effective in their tissue protective functions. Such modified EPOs have the benefit of providing tissue protection without the effect of increasing erythropoiesis, and therefore are suitable for high dose administration or chronic administration often required for tissue protection – for example, for administration to stroke victims – while avoiding the risk of thrombosis associated with administration of wild-type EPO. This concept was demonstrated with *in vitro* and *in vivo* experiments that showed that EPO muteins, particularly those with amino acid substitutions in the domains needed for binding to the Classical EPO Receptor, and EPOs in which the amino acid side chains have been masked by chemical modifications, are both nonerythropoietic and tissue protective in various animal disease models.

The outstanding rejections under 35 U.S.C. § 112, first paragraph, for scope of enablement, made in the Office Action dated December 31, 2007 (the "Office Action") were discussed. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. The possibility of amending

the claims to recite EPOs with mutations in specific regions that affect binding to the classical EPO-R homodimers was discussed.

In view of this discussion, the Examiners indicated that claims reciting EPO muteins having the structural characteristics of having mutations in the regions needed for receptor homodimer binding but not in the remainder of the molecule and the functional characteristics of exhibiting tissue protective activity would be considered if excessive searches were not required.

The Examiners also indicated that they might reconsider the species election set forth in the restriction requirement, mailed June 20, 2006. Specifically, the Examiners indicated that they would consider examining the claims with respect to a greater number of EPO muteins, each mutein capable of binding the Tissue Protective Receptor Complex but not the Classical EPO Receptor, as long as excessive further searches of EPO sequences would not be required.

Applicants also presented results of experiments to support enablement commensurate with the full breadth of the limitation "protecting against and preventing a tissue injury" as well as "restoring and rejuvenating tissue and tissue function in a mammal." The Examiners indicated that the evidence and arguments would be considered if presented.

This Amendment, the evidence presented in the Declaration of Dr. Michael L. Brines, M.D., Ph.D. (the "Brines Declaration") submitted herewith, and the remarks herein reflect the discussion during the Interview.

II. SUBSTITUTE SEQUENCE LISTING

A Substitute Sequence Listing is submitted herewith to correct the Sequence Listing, which lists sequences of the full-length EPO precursor, which includes a leader sequence, whereas the specification discloses amino acid positions of the mature EPO protein, which lacks the leader sequence.

The Substitute Sequence Listing is amended so that SEQ ID NOs:4, 10, and 15-119 represents mature EPO sequences rather than full-length EPO sequences. SEQ ID NO:6 in the Substitute Sequence Listing now represents the full-length EPO precursor.

No new matter is added by this Substitute Sequence Listing, which is supported by the specification as filed. The specification as filed discloses that the first 27 amino acid residues of full-length EPO is a leader sequence (see p. 90, ll. 4-10) and it was well known in the art that mature EPO is produced by the removal of this 27-amino acid leader sequence. See, *e.g.*, Jacobs K *et al.* 1985. "Isolation and characterization of genomic and cDNA clones of human erythropoietin," *Nature* 313(6005):806-810; reference C173 in the Supplemental Information Disclosure Statement submitted concurrently herewith. The specification, however, refers to amino acid positions in mature EPO, but misidentifies them with sequence identifiers that represent full-length EPO (see, *e.g.*, p. 4, ll. 24-25; p. 90, ll. 4-10; and SEQ ID NO:10 of the specification as filed, which states that the sequence TKVNFYAW is at amino acid positions 44-51, but shows that this sequence is located in full-length EPO at positions 71-78). Thus, one of skill in the art reading the specification would understand that the EPO amino acid positions in the specification refer to mature EPO. Therefore, no new matter has been added with the Substitute Sequence Listing.

The Substitute Sequence Listing also contains a correction to the listing of the sequences in response to the Examiner's objection that SEQ ID NO:62 and SEQ ID NO:5 are the same. The sequence identified by SEQ ID NO:5 has been replaced with the sequence formerly identified as SEQ ID NO:212. Applicants note that SEQ ID NO:6 and SEQ ID NO:44 are also identical in the Sequence Listing as filed. In order to correct this duplication, the sequence identified by SEQ ID NO:6 has been replaced with the sequence formerly identified as SEQ ID NO:10 (full-length EPO) in the Substitute Sequence Listing.

III. REQUEST FOR RECONSIDERATION OF RESTRICTION REQUIREMENT AND SPECIES ELECTION

As currently amended, claims 54 and 57 and their dependent claims thereon are limited with respect to the number of mutein recombinant tissue protective cytokines (hereinafter referred to as "EPO muteins") that fall within their scope. In view of this amendment, Applicants respectfully request that the Examiner reconsider the finality of the restriction requirement and the species election of a single EPO point mutation. In particular, Applicants request consideration of the full scope of claims 54-58 as amended, and request

that the Examiner withdraw the objection to claims 56-58 as allegedly reciting non-elected subject matter.

Applicants also traverse the Examiner's withdrawal of claims 69 and 70, and request reconsideration of their withdrawal. Applicants submit that these claims are within the scope of their antecedent claim 57. Specifically, claim 57 as currently amended recites a method for "protecting against or preventing a tissue injury" using an EPO mutein. Claims 69 and 70 recite that the EPO mutein is administered before a surgical procedure, which is a specific embodiment of the method of claim 57. See specification *inter alia* at p. 31, ll. 21-30 and p. 64, ll. 17-32. Accordingly, the subject matter of claims 69 and 70 is within the scope of claim 57 and therefore within the scope of the elected subject matter. As such, it is believed that claims 69 and 70 would not require additional searches. Applicants therefore respectfully request that the Examiner consider claims 69 and 70.

IV. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR LACK OF ENABLEMENT, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. In particular, the Examiner contends that the specification does not reasonably provide enablement across the full scope of the claims. The Examiner acknowledges that the art recognizes protective effects of four different EPO muteins in ischemic injury models and certain inflammatory conditions, but contends that the art and the specification fail to provide guidance or sufficient scientific basis to use any tissue protective cytokine for any tissue injury (Office Action, p. 5). The Examiner concludes that undue experimentation would be required to practice the claimed invention based on the application of the *Wands* factors: "breadth of the claims encompassing the use of molecules with no precise structural requirements, the lack of adequate guidance . . . supporting a therapeutic effect of EPO molecules on the broadly claimed chronic and/or degenerative diseases or disorders, or guidance on their use, the unpredictability in the art . . . and the complex nature of the invention" (Office Action, ¶ bridging pp. 8-9).

Applicants believe that amended claims 54-58 and new claims 71-73 are enabled over their entire scope. Each of these claims recites a method comprising exposing cells, tissue, or organs to a particular EPO mutein. One of skill in the art can readily practice the claimed

invention over its entire scope without undue experimentation. The claims recite a defined class of EPO muteins with amino acid substitutions at a limited number of amino acid residues. The claims further provide a clear-cut test that can be used by the skilled artisan to determine whether any particular EPO mutein within this defined class has the required activity, *i.e.*, tissue protection but reduced erythropoietin activity. Thus, a skilled person can readily determined the limited number of EPO muteins encompassed buy the claims, and use them to practice the invention without undue experimentation.

1. EPO muteins

Claims 54-58 have been amended to recite methods using specific EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor, *i.e.*, amino acids at positions 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1]; 44 to 51 of SEQ ID NO:10 [SEQ ID NO:2]; 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3]; and 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4] (see specification at, *e.g.*, p. 40, ll. 2-7). Because these EPO muteins have amino acid substitutions in regions that affect binding to the Classical EPO Receptor, they have reduced erythropoietic activity. However, since the amino acids in these regions do not affect binding to the Tissue Protective Receptor Complex, these EPO muteins maintain their tissue protective function. See Brines Declaration, ¶¶ 23-24.

The claims have also been amended to recite specific, art-recognized tests that require that the EPO muteins have reduced erythropoietic activity yet maintain tissue protective activity. In particular, the claims require that the EPO muteins have (i) a reduced level of *in vivo* erythropoietic activity compared to native erythropoietin as determined by the exhypoxic polycythemic mouse bioassay and (ii) tissue protective activity *in vivo* as determined by the middle cerebral artery occlusion test or *in vitro* as determined by the P19 assay.

As such, the claims as amended recite a limited class of EPO muteins having structural and functional features necessary to provide tissue protection while having a reduced ability to stimulate erythropoiesis and are not directed to <u>any</u> tissue protective cytokine.

The amended and new claims are enabled by the specification's extensive teaching of methods to make the claimed EPO muteins and test them for erythropoietic and tissue protective activity. See Brines Declaration, ¶¶ 5 and 18-22. Additional such assays were well-known in the art as of the filing date of the instant application. See Brines Declaration, *id.* Indeed, several such muteins have been made and tested for erythropoietic activity and tissue protective activity in accordance with these teachings, as demonstrated by the working examples of the specification and experiments conducted after the filing date of the instant application (see explained in the Brines Declaration, ¶¶ 5-12), as described below.

Amino acids 100-108. As examples of EPO muteins with amino acid substitutions at positions 100 to 108 of SEQ ID NO:10 [SEQ ID NO:3], the specification provides working examples that show that EPO muteins with substitutions in this region are tissue protective and have reduced erythropoietic activity compared to wild-type recombinant EPO. For example, the EPO muteins S100E and R103E have reduced erythropoietic activity (see specification, Example 17 at p. 126, l. 12 to p. 128, l. 13) but are tissue protective. In particular, the specification teaches that S100E maintains the viability of neuroblastoma cells against rotenone in an *in vitro* assay (Example 3 at page 102, ll. 5-23). The specification further teaches that S100E is protective in well-established assays for apoptosis, for example, protection of P19 cells from apoptosis following serum withdrawal (Example 15 at p. 124, l. 1 to p. 125, l. 2), protection of primary hippocampal neuron cell cultures from NMDA-induced cell death (Example 14 at p. 122, ll. 30 to p. 123, l. 30), and protection of neuronal-like cells from cell death upon withdrawal of nerve growth factor (Example 16 at p. 125, l. 5 to p. 126, l. 6). See also Brines Declaration, ¶¶ 8-9. In addition, the mutein R103E has been shown to reduce NMDA-induced apoptosis of rat hippocampal neurons *in vitro*. See Brines Declaration, ¶ 9. Thus, in well-established *in vitro* assays for apoptosis using different cell types, muteins substituted in amino acid positions 100-108 were shown to be cell-protective.

These *in vitro* results have been corroborated by *in vivo* studies. Example 12 of the specification shows that the S100E mutein reduces functional deficits in motor neurological function in an animal model of traumatic spinal cord injury (specification at p. 115, ll. 21-30 and p. 116, l. 20 to p. 117, l. 5). The S100E mutein and the R103E mutein are tissue protective in retina, shown using an animal model of glaucoma. See Example 18 of the

specification at p. 128, l. 17 to p. 129, l. 8. See also Brines Declaration, ¶ 10. In a more recent study, the mutein S100E was demonstrated to improve neurological function after stroke. In another experiment, S100E and R103E were found to prevent injury that was caused by sciatic nerve compression in rats. See Brines Declaration, ¶¶ 11-12.

The specification also teaches that the double substitution EPO mutein K45D/S100E is tissue-protective in an *in vivo* animal model of glaucoma. See Example 18 of the specification at p. 128, l. 17 to p. 129, l. 8. Moreover, the double substitution mutein K45D/S100E has been demonstrated to prevent injury due to sciatic nerve compression in rats. See Brines Declaration, ¶ 12.

Amino acids 146-151. As an example of an EPO mutein with amino acid substitutions at positions 146 to 151 of SEQ ID NO:10 [SEQ ID NO:4], the specification teaches that the EPO mutein R150E prevents NMDA-induced cell death in primary hippocampal neuron cell cultures. See specification, Example 14 at p. 122, ll. 30 to p. 123, l. 30. Moreover, in a subsequent study, R150E was found to prevent P19 cell apoptosis. See Brines Declaration, ¶ 9. Again, these *in vitro* results were confirmed *in vivo*. For instance, the specification provides a working example to demonstrate that R150E is tissue protective in retina in an *in vivo* animal model of glaucoma. See specification, Example 18 at p. 128, l. 17 to p. 129, l. 8.

Chemically-modified amino acids. EPO muteins with amino acid substitutions in four regions that affect binding to the Classical EPO Receptor have reduced erythropoietic activity but maintain their tissue protective function. A similar effect is achieved when the charge of amino acids in these four regions is altered by chemical modification. See Brines Declaration, ¶ 13. Indeed, the specification teaches several chemical modifications of the EPO molecule that result in EPOs with tissue protective activity but reduced erythropoietic activity. Such modifications include modification of arginine residues with a vicinal diketone (*e.g.*, cyclohexanedione) or R-glyoxal (*e.g.*, phenylglyoxal), modification of tyrosine residues by nitration (using, *e.g.*, a trinitrophenyl compound) or iodination, modification of lysine residues by, *e.g.*, carbamylation, guanidination, or carboxymethylation, and oxidation of tryptophan residues by succinylation. See specification at p. 52, l. 1 to p. 53, l. 25; p. 54, ll. 8-10; p. 55, ll. 13-18; p. 109, ll. 23-28; Example 4 at p. 104, ll. 11-22 and p. 105, l. 23 to p.

107, l. 10; Example 5 at p. 108, l. 19 to p. 109, l. 16; Example 6 starting at p. 111; Example 12 at p. 117, l. 6 to p. 118, l. 15.

EPOs with chemically-modified lysines (EPOs that have been carbamylated, carboxymethylated, PEGylated, carbamylated and PEGylated, or succinylated), arginines (by cyclohexanedionation and phenylglyoxalation), glutamate/aspartate (EDC-ethanolamination), and tyrosines (trinitrophenylation), and EPOs in which the disulfides are reduced by iodoacetamidation, have been shown to be nonerythropoietic yet tissue protective in various tissues and disease or injury conditions. See Brines Declaration, ¶¶ 13-17.

Accordingly, if an EPO with a chemically-modified arginine, for example, in a domain that affects Classical EPO Receptor binding (*i.e.*, a chemical modification of the arginine at position 14, 103 or 150) retains its tissue protective function, it would follow that an EPO having a single substitution of an arginine at that same position would also be tissue protective. This is indeed the case, as described *supra* for the R103E and R150E muteins. Therefore, the experiments showing a tissue protective function for EPOs with chemically-modified amino acids at particular positions indicate that EPOs with mutations in amino acids at those positions – in particular, amino acids 11-15 of SEQ ID NO:10 (VLQ**RY**), amino acids 44-51 (T**K**VNF**Y**A**W**), 100-108 (SGL**R**SLTTL), and 146-151 (SNFL**R**G) (residues that have been modified by chemical modification are shown in bold/underline) – are also tissue protective.

2. Protection or Rejuvenation of Tissues

The Examiner acknowledges that routine technology and bioassays can be used to make the claimed EPO muteins, but contends that the testing of numerous mutants for tissue protection would require undue experimentation, because tissue responsiveness will vary depending on the mutein, different mutein doses must be tested, and *in vitro* testing does not parallel the *in vivo* protection, wherein the injury can arise due to various causes (Office Action, p. 8). Applicants respectfully submit that practicing the claimed invention does not require undue experimentation.

The methods of the present claims are used to prevent and treat conditions that result from cell death and subsequent tissue damage induced by inflammation and oxidative

damage. Because the Tissue Protective Receptor Complex is present on a large variety of tissues, and EPO muteins with reduced erythropoietic function are tissue protective in a broad range of diseases and injuries, EPO muteins are able to confer tissue protection in a broad range of tissues and for a broad range of diseases and injuries. Thus, an EPO mutein found to have tissue protective activity in the *in vitro* or *in vivo* assays recited in the claims can be used in the claimed methods regardless of the source of the injury, other symptoms of the disease or condition, and the cell type affected. See Brines Declaration, ¶¶ 23-28.

Therefore, the skilled artisan would be able to practice the claimed invention with a limited amount of experimentation using routine, art-accepted methods. This amount of experimentation is well within the legal standard, which does not preclude a certain amount of experimentation and unpredictability of the results. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); *In re Angstadt*, 190 U.S.P.Q. 214, 219 (C.C.P.A. 1976); *In re Neuberger & Rabbitts,* 2002 WL 33952578 (B.P.A.I. 2002)(Board reversed Examiner's non-enablement rejection of claims to a biotechnological invention because "a requirement for certainty would be incompatible with any amount of experimentation and therefore incompatible with the standard of enablement." *Id.* at 3). All that is required is a reasonable amount of guidance with respect to the direction of the experimentation; reasonable certainty with regard to the *outcome* of the experimentation is *not* required.

Moreover, the skilled artisan would understand that a certain amount of experimentation and optimization is required to determine the efficacy (whether using a cell line or experimental animal or in a patient) of a particular EPO mutein. Indeed, the optimal dosage *for any* pharmaceutical composition must be decided according to the judgment of the practitioner and each patient's circumstances, and the dose is determined based not only on the particular EPO mutein but also on the condition or disease to be treated, the body mass of the patient, the route of administration, *etc.* See specification at p. 77, ll. 8-20. As stated in *In re Brana*, 5 F.3d 1557, 34 U.S.P.Q.2d 1437, 1442 (Fed. Cir. 1995), the testing for the full safety and *effectiveness* of a product is more properly left to the Food and Drug Administration and the requirements under the law for obtaining a patent should not be confused with the requirements for obtaining government approval to market a particular drug or therapeutic method for public use.

Thus, based on the applicable case law and the teachings of the specification, Applicants do not believe that the need to determine the optimal dosage of a mutein is a proper basis for an allegation of undue experimentation.

Furthermore, Applicants also disagree with the Examiner's contention that *in vitro* testing does not parallel the *in vivo* protection. Indeed, the tissue protection obtained in *in vitro* and *in vivo* assays with specific EPO muteins described *supra* provides ample evidence to the contrary. Tissue protection *in vitro* is predictive of tissue protection *in vivo*, as demonstrated by the working examples of the instant specification and explained in the Brines Declaration. See Brines Declaration, ¶¶ 18-20.

Finally, the Examiner acknowledges that EPO muteins spare neuronal tissue loss due to injury and reduce cell death, but states that it would be necessary to "implicate EPO as being able to induce *new cellular growth*, which would be necessary to restore or rejuvenate tissue" (Office Action, ¶ bridging pp. 5-6; emphasis added). Applicants respectfully disagree that a demonstration that EPO can induce new cellular growth is required to "restore" or "rejuvenate" tissue.

The term "restore" in the claims is understood to mean (1): give back, return; and (2): to put or bring back into existence or use (definitions (1) and (2) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 1005, entry for "restore" (Exhibit B)). In the context of restoring function, meanings (1) and (2) are appropriate where a mutein may be used to "give back" function to a cell, tissue, or organ that had lost function, *e.g.*, as a result of injury or trauma, or where a mutein may be used to put a cell, tissue, or organ "back into use." By analogy, a work of art or a house may be "restored," but it is never fully returned to its original state or condition. Likewise, Applicants submit that the ordinary meaning of the term "restore," as it is used in the instant claims, does not require new cell growth or even that the claimed cells, tissues, and organs are fully returned to their original state or regain full function.

Likewise, "rejuvenate" means: (a) to make young or youthful again: reinvigorate; (b): to restore to an original or new state (definition (1) from Webster's Ninth New Collegiate Dictionary, Springfield, MA: Merriam-Webster, Inc., 1986, p. 994, entry for "rejuvenate"

(Exhibit B)). In the context of rejuvenating tissue or tissue function, this definition is appropriate where a mutein may be used to "reinvigorate" a tissue that had lost function, for example, as a result of injury or trauma, or where a mutein may be used to "restore" (as defined in the preceding paragraph) a tissue – not necessarily to its original state, but to *a new, functional state*. As such, Applicants submit that the ordinary meaning of the term "rejuvenate," as it is used in the instant claims, does not require new cell growth but only that the function of the tissue or organ is restored.

These definitions of restore and rejuvenate are consistent with the teachings of the specification. For example, the specification provides an experiment in which mice were subjected to a brain trauma that impairs cognitive function and then treated with EPO. The mice that received EPO demonstrated an improvement of cognitive function – thus, the treated mice had restoration of brain function. See Example 10 of specification starting at p. 114. Therefore, the meaning of the terms "restore" and "rejuvenate" as used in the specification do not require new cell growth.

Based on the above, claims 54-58 satisfy the enablement requirement. Applicants therefore respectfully request the withdrawal of the rejection of claims 54-58 under 35 U.S.C. § 112, first paragraph, for lack of enablement. Applicants further submit that, for the reasons set forth above, new claims 69-73 are also enabled.

V. THE REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION, SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants believe that the amendment of claims 54, 56 and 57 obviates this rejection for the reasons set forth below.

Specific mutein recombinant tissue protective cytokines that may be used in the claimed invention are described throughout the specification. For example, particular muteins within the scope of the claims as amended are provided at, *e.g.*, pp. 32-35 and 47-49 of the specification. Examples of specific EPO muteins that are reduced for erythropoietic activity compared to EPO are provided in the working examples of the specification (see Example 17). The tissue protective function of EPO muteins is also demonstrated by

working examples. See specification, Example 3 (S100E), Example 11 (S100E), Example 14 (R130E and R150E), Example 15 (S100E), Example 16 (S100E), and Example 18 (R103E, R150E, S100E).

Moreover, the instant specification discloses a correlation between the structure of the claimed muteins and their function. Specifically, the regions now defined in the claims affect erythropoietic activity of EPO. Mutations in one or more amino acids in this area yields a mutein having reduced erythropoietic activity compared to native recombinant human EPO. See specification at p. 4, l. 20 to p. 5, l. 2.

Therefore, the specification provides extensive description of the EPO muteins recited in the claims, methods for their use, and numerous working examples that demonstrate the correlation between the between structure of the EPO muteins and their function, such that a skilled artisan would recognize that Applicants were in possession of the claimed invention at the time of filing. Thus, the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description should be withdrawn.

VI. THE REJECTIONS UNDER 35 U.S.C. § 102 SHOULD BE WITHDRAWN

Claims 54-58 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by International Application Publication No. WO 02/053580 to Brines *et al.*, filed on December 28, 2001. Applicants submit that International Application Publication No. WO 02/053580 does not anticipate the claims as amended because this reference generically discloses EPO muteins, but does not disclose the specific EPO muteins recited in the instant claims. This reference does not list the specific muteins recited in the instant claims, *i.e.*, EPO muteins with the particular structural and functional features required by the claims, such as mutations in 28 amino acids of the 165 amino acids of the mature protein. See specification at p. 39, ll. 9-12. Therefore, the broad genus of "mutein" disclosed in these references does not anticipate the specific, defined mutein species of the claims as amended. *Cf. Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, 468 F.3d 1366 (Fed. Cir. 2006) (holding that a prior patent containing a formula including hundreds of compounds does not anticipate the specific compound of the claims, and the specific compound was not defined in the prior patent).

Accordingly, amended claims 54-58 are not anticipated by WO 02/053580 and Applicants respectfully request the withdrawal of this rejection.

Claims 54-58 are rejected under 35 U.S.C. § 102(b), as allegedly being anticipated by Campana *et al.*, 1998, *Int J Mol Med* 1(1):235041; abstract ("Campana"). Applicants believe that this rejection is in error. The EPO muteins of amended claims 54-58 require a substitution of an amino acid residue at one or more specific amino acid positions of the EPO protein. In contrast, Campana discloses a 17-mer EPO peptide, which could be generated by deletion of amino acids from the EPO sequence. Since the claims recite EPO proteins with alterations at specific amino acid positions, and the Campana peptide does not have any substituted amino acids, Campana does not anticipate the claims. Moreover, the claimed methods for protecting, maintaining or enhancing the viability of cells, tissues, or organs, treating or preventing diseases, and restoring or rejuvenating tissues or tissue function are not taught by Campana.

Therefore, Applicants respectfully request the withdrawal of the rejection of claims 54-68 for anticipation by Campana under 35 U.S.C. § 102(b).

VII. THE CLAIM REJECTIONS FOR DOUBLE PATENTING SHOULD BE CONTINUED TO BE HELD IN ABEYANCE

Claim 57 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 of U.S. Patent No. 6,531,121 B2.

Claims 54-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 35 and 37-38 of copending Application No. 10/188,905.

Claims 57-58 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 15 of copending Application No. 09/716,960.

Claims 54-56 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2 and 5 of copending Application No. 10/351,640.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 10/185,841.

Claim 57 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 4 of copending Application No. 10/573,905.

In response Applicants request that the double patenting rejections continue to be held in abeyance until indication of allowable subject matter in the present application.

CONCLUSION

Entry of the foregoing amendments and remarks into the record of the above-identified application is respectfully requested. Applicants estimate that the remarks made herein place the pending claims in condition for allowance.

Respectfully submitted,

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Enclosures

Appendix A: Replacement table for pages 69-71 of the specification

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
Heart	Ischemia	Coronary artery disease	Acute, chronic Stable, unstable
		Myocardial infarction	Dressler's syndrome
		Angina	
		Congenital heart disease	Valvular Cardiomyopathy
		Prinzmetal angina	
		Cardiac rupture	Aneurysmatic Septal perforation
		Angiitis	
	Arrhythmia	Tachy-, bradyarrhythmia *Supra*ventricular, ventricular Conduction abnormalities	Stable, unstable Hypersensitive carotid sinus node
	Congestive heart failure	Left, right, bi-ventricular, systolic, diastolic	Cardiomyopathies, such as idiopathic familial, infective, metabolic, storage disease, deficiencies, connective tissue disorder, infiltration and granulomas, neurovascular
		Myocarditis	Autoimmune, infective, idiopathic
		Cor pulmonale	
	Blunt and penetrating trauma		
	Toxins	Cocaine toxicity	
Vascular	Hypertension	Primary, secondary	
	Decompression sickness		
	Fibromuscular hyperplasia		
	Aneurysm	Dissecting, ruptured, enlarging	
Lungs	Obstructive	Asthma Chronic bronchitis, Emphysema and airway obstruction	
	Ischemic lung disease	Pulmonary embolism, Pulmonary thrombosis, Fat embolism	
	Environmental lung diseases		
	Ischemic lung disease	Pulmonary embolism Pulmonary thrombosis	
	Interstitial lung disease	Idiopathic pulmonary fibrosis	
	Congenital	Cystic fibrosis	
	Cor pulmonale		
	Trauma		

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Pneumonia and pneumonitides	Infectious, parasitic, toxic, traumatic, burn, aspiration	
	Sarcoidosis		
Pancreas	Endocrine	Diabetes mellitus, type I and II	Beta cell failure, dysfunction Diabetic neuropathy
		Other endocrine cell failure of the pancreas	
	Exocrine	Exocrine pancreas failure	pancreatitis
Bone	Osteopenia	Primary secondary	Hypogonadism immobilisation Postmenopausal Age-related Hyperparathyroidism Hyperthyroidism Calcium, magnesium, phosphorus and/or vitamin D deficiency
	Osteomyelitis		
	Avascular necrosis		
	Trauma		
	Paget's disease		
Skin	Alopecia	Areata Totalis	Primary Secondary Male pattern baldness
	Vitiligo	Localized generalized	Primary secondary
	Diabetic ulceration		
	Peripheral vascular disease		
	Burn injuries		
Autoimmune disorders	Lupus erythematodes, Sjiogren, Rheumatoid arthritis, Glomerulonephritis, Angiitis		
	Langerhan's histiocytosis		
Eye	Optic neuritis		
	Blunt and penetrating injuries, Infections, Sarcoid, Sickle C disease, Retinal detachment, Temporal arteritis		

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Retinal ischemia, Macular degeneration, Retinitis pigmentosa, Arteriosclerotic retinopathy, Hypertensive retinopathy, Retinal artery blockage, Retinal vein blockage, Hypotension, Diabetic retinopathy, and Macular edema		
Embryonic and fetal disorders	Asphyxia		
	Ischemia		
CNS	Chronic fatigue syndrome, acute and chronic hypoosmolar and hyperosmolar syndromes, AIDS Dementia, Electrocution		
	Encephalitis	Rabies, Herpes	
	Meningitis		
	Subdural hematoma		
	Nicotine addiction		
	Drug abuse and withdrawal	Cocaine, heroin, crack, marijuana, LSD, PCP, poly-drug abuse, ecstasy, opioids, sedative hypnotics, amphetamines, caffeine	
	Obsessive-compulsive disorders		
	Spinal stenosis, Transverse myelitis, ~~Guillian~~ Guillain Barre, Trauma, Nerve root compression, Tumoral compression, Heat stroke		
ENT	Tinnitus Meuniere's syndrome Hearing loss		
	Traumatic injury, barotraumas		
Kidney	Renal failure	Acute, chronic	Vascular/ischemic, interstitial disease, diabetic kidney disease, nephrotic syndromes, infections, injury, contrast-induced, chemotherapy-induced, CPB-induced, or preventive
	Henoch S. Purpura		
Striated muscle	Autoimmune disorders	Myasthenia gravis Dermatomyositis Polymyositis	

Cell, tissue or organ	*Dysfunction or pathology*	*Condition or disease*	*Type*
	Myopathies	Inherited metabolic, endocrine and toxic	
	Heat stroke		
	Crush injury		
	Rhabdomylosis		
	Mitochondrial disease		
	Infection	Necrotizing fasciitis	
Sexual dysfunction	Central and peripheral (e.g. erectile dysfunction)	Impotence secondary to medication, (diabetes)	
Liver	Hepatitis	Viral, bacterial, parasitic	
	Ischemic disease		
	Cirrhosis, fatty liver		
	Infiltrative/metabolic diseases		
Gastrointestinal	Ischemic bowel disease		
	Inflammatory bowel disease		
	Necrotizing enterocolitis		
Organ transplantation	Treatment of donor and recipient		
Reproductive tract	Infertility	Vascular Autoimmune Uterine abnormalities Implantation disorders	
Endocrine	Glandular hyper- and hypofunction		